



## **Amine Quantum Dots Conjugation Kits**

### **(Catalog # OAK)**

Ocean NanoTech's amine functionalized Quantum Dots (QDs) are fluorescent nanocrystals with a high density of amine groups on the surface. The QDs can be used to specifically conjugate thiol containing ligands/protein/antibody with low non-specific binding. The kit contains sufficient reagents & components for performing 4 conjugation reactions using 0.25 nmoles QDs per reaction.

Briefly, the QDs are activated using sulfo-SMCC (sulfo-succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate) followed by conjugation to thiol groups present on the protein/ligands, which are generated from primary amine groups via Traut's Reagents. The protocol shown below has been used successfully conjugate bovine serum albumin, streptavidin, and immunoglobulin to Ocean NanoTech's QDs.

**IMPORTANT: PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING.**

#### **Amine QDs Conjugation Kits (Catalog # OAK) contents:**

- QDs (Catalog# QSA), 8  $\mu$ M, 0.125 mL.
- CB300: Coupling Buffer, 15 mL.
- QB300: Quenching Buffer, 0.5 mL.
- SB300: Storage Buffer, 15 mL.
- Sulfo-SMCC, 20 mg sulfo-SMCC (**store at -20 °C upon arrival**).

#### **Materials required but not provided:**

- Target protein/ligand with thiol groups
- Pipettes for delivering 10  $\mu$ L to 1 mL volumes
- Vortex mixer capable of securing 1.5 mL tubes for incubations
- Standard laboratory disposables
- -20 °C freezer and 4 °C refrigerator
- NAP-5 desalting column (Product#17-0853-02, GE Healthcare Life Sciences)
- DMSO (Dimethyl sulfoxide)

#### **Reagents Preparation:**

*NOTE: Allow all reagents to come to room temperature before starting.*

##### **Protein/Ligand Solution:**

Each conjugation needs 0.125 mg protein with thiol groups for 0.25 nmole QDs. Disulfide bonds in proteins can be reduced to thiol groups by DTT or TCEP. Primary amine groups on proteins also can be modified to thiol groups *via* Traut's Reagents.

Thiol groups can be added to peptides during synthesis processes.

Dissolve/dilute protein/ligand with thiol groups to 1 mg/ml in Coupling Buffer.

▪ **Sulfo-SMCC**

Weigh out 1.5 mg of sulfo-SMCC and add 0.15 mL DMSO into the tube and mix well to dissolve the solids. The resulting concentration for sulfo-SMCC is 10 mg/mL.

*Note: The sulfo-SMCC is not stable in the aqueous solution. Each sulfo-SMCC solution should be prepared only before immediate use and is good for one reaction only. After an aliquot of the sulfo-SMCC solution, do not use the remaining sulfo-SMCC solution.*

**Conjugation Protocol:**

*NOTE: It is best to use plastic low protein binding microcentrifuge tubes of at least 1.5 mL capacity to perform all reaction.*

1. Aliquot 31.25  $\mu$ L of QSA (8  $\mu$ M) into a microcentrifuge tube with 31.25  $\mu$ L Coupling buffer and mix well.
2. Add 20  $\mu$ L of sulfo-SMCC (10 mg/mL) to the QD solution. Mix well.
3. React at room temperature for 1 hr with continuous mixing.
4. Load all activated QDs to the NAP-5 column equilibrated with Coupling Buffer. Add 0.3 mL Coupling Buffer to the column after all nanoparticle solution enter the column. Collect eluted solution (0.3 mL) into a tube labelled as activated QD.
5. Add 0.125 mg of thiolated protein to the activated QD solution. React at room temperature for 2 hrs with continuous mixing.
6. Add 10  $\mu$ L Quenching Buffer to the solution. Mix well and incubate for 30 mins at room temperature with continuous mixing.
7. Purify the protein conjugated QD with Washing/Storage Buffer two rounds by ultrahigh speed centrifugation (**Table 1**).
8. Resuspend QD conjugates in Washing/Storage buffer to get desired concentration.

**Table 1. Suggested ultracentrifugation speed for QD conjugate purifications**

Wavelength (nm)	Relative centrifugal field for ultracentrifugation (xg)	Time
425	350 k	45-75 minutes
525	325 k	45-75 minutes
540	325 k	45-75 minutes
560	300 k	45-75 minutes
580	275 k	15-45 minutes
600	250 k	15-45 minutes
620	200 k	15-45 minutes
645	125 k	15-45 minutes
665	100 k	15-45 minutes

**Storage:**

- All the solutions in the kit should be stored at 4°C. The sulfo-SMCC vial should be stored at -20 °C.
- The conjugates can be stored in the Washing/Storage Buffer at 4 °C.

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